

## I. Scientific Abstract

This human gene therapy protocol proposes to examine the safety of administering autologous ex-vivo expanded CD8<sup>+</sup> cytotoxic T lymphocyte (CTL) clones genetically modified to express a CD20-specific chimeric immunoreceptor (scFvFc:ζ) to patients with recurrent or refractory CD20<sup>+</sup> non-Hodgkin's lymphoma (NHL) undergoing a standard myeloablative preparative regimen with stem cell rescue. T cells present in peripheral blood mononuclear cells (PBMC) isolated from study subjects prior to transplantation will be polyclonally activated with anti-CD3 antibody OKT3 then genetically modified by electroporation with linearized plasmid DNA vector encoding the CD20-specific scFvFc:ζ and the NeoR genes under the transcriptional control of the CMV immediate/early and SV40 promoters, respectively. Genetically modified T cell clones generated from this procedure will be evaluated for cell surface phenotype by flow cytometry, chromosomal integration status of plasmid vector by Southern blot, receptor expression by Western blot, and CD20-specific effector function by chromium release assay. Clones meeting all quality-control criteria will be expanded by recursive stimulations with OKT3/rhIL-2 in the presence of irradiated feeder cells. Beginning twenty-eight days after stem cell rescue, patients will receive a series of three escalating cell dose infusions at two week intervals of their genetically-modified CD20-specific CD8<sup>+</sup> CTL clones. Each patient will be evaluated to establish the safety of this procedure with increasing cell doses of 10<sup>8</sup> cells/m<sup>2</sup>, 10<sup>9</sup> cells/m<sup>2</sup>, and 10<sup>10</sup> cells/m<sup>2</sup> of body surface area. The secondary objectives of this protocol are to study the *in vivo* persistence of transferred cells by Q-PCR of peripheral blood samples for vector-specific sequence and to document the trafficking of transferred cells to lymph nodes by *in situ* T-FISH with vector specific probes. When possible, the anti-lymphoma activity of infused clones will be assessed by standard radiographic and clinical follow-up as well as by quantitating levels of circulating CD20<sup>+</sup> B-cells by flow cytometry in the post-transplant period. Additionally, patient peripheral blood samples will be obtained following adoptive therapy and evaluated for evidence of humoral and cellular immune reactivity against the infused genetically-modified clones. This pilot phase I study will serve to provide safety data to support the initiation of larger Phase I/II clinical trials in lymphoma patients utilizing adoptively-transferred CD20-specific CD8<sup>+</sup> CTL in conjunction with infusional IL-2 and/or co-infusion of CD20-specific T<sub>H1</sub> CD4<sup>+</sup> clones.